1B.2 Physiological field evaluation for drought tolerance.Field phenotyping methods for the development of the new synthetic population

Alain Audebert, Cécile Grenier, Marc Châtel, Yolima Ospina, Francisco Rodriguez

Funding: Cirad, CIAT Cooperators

Abstract

The Cirad/CIAT collaborative project has developed a breeding program based on the recurrent selection (RS) for an eco-efficiency use of water. Selection was primarily based on yield and, in the course of the selection process, screening methods were refined with additional secondary relevant traits (morpho-pheno-physiological traits). The infrared (IR) thermography measures the plant canopy temperature, which gives an indication of the plant water status through leaf surface cooling capacity by transpiration along environmental conditions. We developed large scale phenotyping methods, adapting the IR technology to field conditions, to evaluate response to drought among synthetic populations.
An experiment was conducted during the 2008/2009 dry season in the Santa Rosa CIAT research station in Villavicencio (Colombia) to adjust the phenotyping method based on IR thermography and to evaluate 400 S_1 lines and 1000 S_0 plants derived from synthetic populations. This evaluation method allowed us to identify interesting progenitors to be integrated in the population improvement scheme with the goal to develop and diffuse improved material, populations or lines, with increased resilience to drought for various rainfed ecosystems in Latin America and the Caribbean (LAC).

Background

Enhanced crop production under limited water supply depends on a subtle dosage of various physiological mechanisms and plant traits according to timing, intensity and duration of the water deficit period. Unfortunately, knowledge on plant response to water deficit has only poorly impacted genetic improvement for crop productivity in drought-prone area. Indeed, the method based on direct selection for grain yield still produces the best-performing genotypes, particularly for rice. Facing this situation, the Cirad/CIAT rice collaborative project intends to develop populations, lines and methodological tools to assist in the selection process. Integration of expertise from Cirad and CIAT on rice RS and plant physiology under water stress is expected to provide significant headway for selecting germplasm with better adaptation or resilience to drought conditions.

The project aims at creating new rice synthetic population, using available information on phenotypic traits related to drought tolerance in rice. With further knowledge on loci and alleles related to drought response, this population will also be used to develop breeding methods integrating molecular markers and ecophysiological criteria to enhance RS for drought tolerance and water use efficiency. Crop development models will also be used to analyze and predict the behavior of advanced breeding lines in the targeted environments. The improved genetic resources (population and lines) and methods developed in our project will be shared within the LAC rice breeders' network and with other CGIAR and national breeding programs. This new population will remain open to be enriched with new alleles at other target loci as and when available. The expected outputs are (i) new genetic resources (populations with a broad genetic base and advanced lines) with improved drought tolerance or improved water use efficiency, (ii) validated methods of molecular marker and crop model based selection for drought tolerance and (iii) better understanding of the physiological and genetic bases of drought tolerance mechanisms.

The experiment was conducted during the dry off-season 2008-2009 and its specifics objectives were:

-The physiological screening in field conditions of genetic material extracted from four synthetic populations

-Development of phenotyping methods based on IR thermography

Material and Methods

From an original synthetic population created in 1995 with a large set of progenitors (PCT-4) four populations were generated differing for the specific selective pressure and number of recombination cycles they were subjected to (cf. Grenier et al.'s chapter in this report).

A large scale phenotype screening was set-up during the dry off-season 2008-2009 at the Experimental Station of Santa Rosa (EESR) in two experiments. For both experiments, a 15-days water stress was applied after panicle initiation stage, and field screening methods consisted in evaluating canopy temperature with IR thermographic camera (NEC IR Camera) at the end of the water stress period (78-80 DAS).

-Experiment 1: Evaluation of 400 S_1 lines coming from the four synthetic populations previously presented. Two treatments were applied; one with the stress and one, used as a control, where irrigation was maintained during the entire experiment. Evaluations were conducted for both treatments. The experimental set-up followed an augmented split block design. The complete experiment consisted in a total of 32 blocs (16 blocs per treatment) with 25 S_1 lines and six controls varieties within each bloc. The control of environmental variation was achieved through the use of soil moisture monitor with Diviner 2000 equipment (Sentek). The soil moisture was monitored for two of the control varieties; Curinga and Moroberekan.

-Experiment 2: Screening for drought response among a population composed of 1000 individual S_0 plants originating from the population PCT-4\0\0\3. The same six control varieties as in experiment 1 were distributed regularly within the population. As in experiment 1 soil moisture was monitored for Curinga and Moroberekan.

The two experiments received nutrient application with SPT (basal application at 70 kg/ha), KCL (70 kg/ha in 2 application) and N (80 kg/ha of urea in 3 applications). Micronutrients were applied as 20 kg/ha Fertimex.

Results and Discussion

A. Experiment 1
   Leaf temperature at/around flowering stage exhibited strong and significant varietal differences that were negatively correlated with soil moisture content and yield. Figure 1 shows the effect of water stress on canopy temperature. In the Santa Rosa 2008-2009 dry season, the canopy temperature of rice populations under stress condition was in average 3.2°C greater than the in the control treatment.

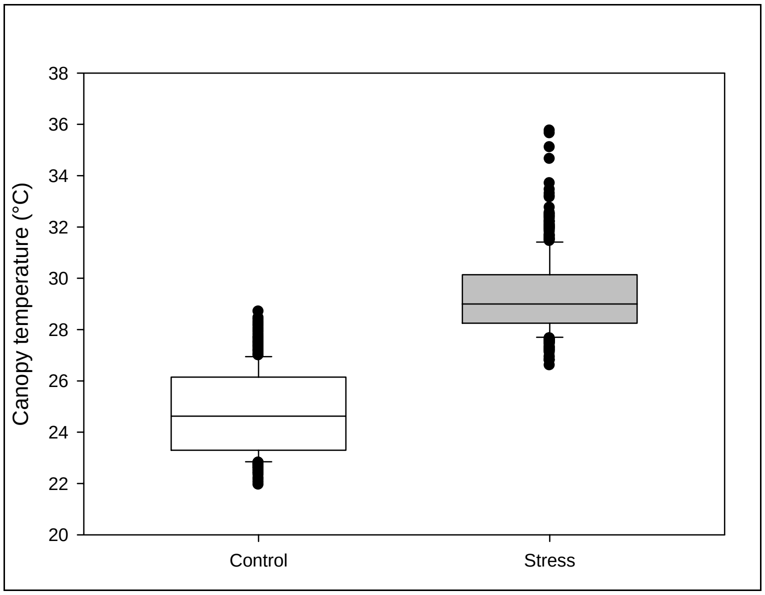

Figure 1: Effect of water deficit on the rice canopy temperature. n= 496 S_1 lines

The frequency histogram obtained for the canopy temperature also shows the existence of genetic variability for this trait (Figure 2). This diversity existed for the control as well as for the stress condition. The canopy temperature among lines did not rank similarly in both treatments. However, particular lines with low canopy temperature in the control treatment also showed a low canopy temperature in the stress treatment. Thus these materials seem to display better transpiration ability in all water availability conditions.

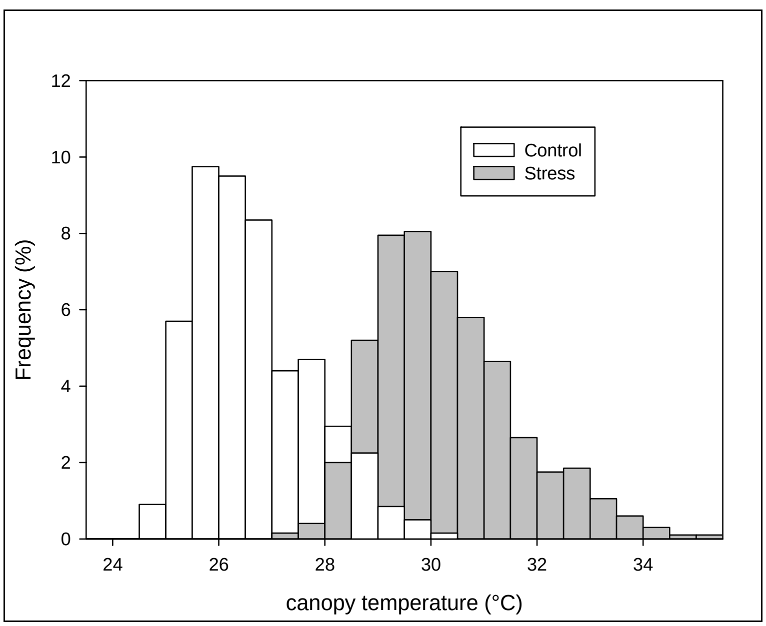

Figure 2: Frequency distribution of canopy temperature for all lines tested and for the two treatments. n = 496 S_1 lines.

This phenotyping approach permitted identifying genotypes with good maintenance of transpiration, and thus sustained growth, under drought stress. Preservation of this capacity to transpire is mainly related to limited water extraction and/or greater root depth. The IR phenotyping also enabled detecting genotypes that have high transpiration rate, and consequently higher potential growth rate, under irrigated control conditions. Selecting suitable combinations of both characteristics is expected to help identifying drought tolerant material with high yield potential.

Seventy five S_1 families were found with low temperature differential between the stress and the control condition, indicating good transpiration potential under water deficit and a certain degree of resilience to drought. Nineteen of these families also showed good agronomic values in a subsequent evaluation of the same 400 lines during the 2009 growing season under non-stress condition in the Experimental Station of La Libertad (Colombia). These 19 lines present valuable traits and could constitute a group of materials on which to concentrate efforts for line advancement and population improvement.

The outcomes of the phenotyping experiment EESR (2008-2009) and the results obtained were reported in the Inter Drought III meeting in Shanghai, China (Audebert et al., 2009).

B. Experiment 2

The use of the IR thermography under water limited conditions also permitted to detect individual plants with lower canopy temperature among a synthetic population. With this method, 16 S_0 plants were found with great potential for response to drought. From these selected plants, eight fertile ones with good seed set were selected to constitute the pool of progenitors to go through recombination for developing a new population. The recombination cycle occurred in Palmira (CIAT-HQ) in 2009 and a bulk of male sterile plants was collected to constitute a new population with improved resilience to drought (PCT-4\EF\1\3). This population will follow the recurrent selection to preserve and enhance its genetic variability and its adaptive characteristic for drought prone environment.

Conclusion

These experiments confirmed the effect of water deficit on canopy temperature. Canopy temperature could thus be used as a criterion to screen for drought tolerance and to serve as a selection tool for breeding programs. Furthermore, the frequency histogram proved the existence of genetic diversity for this trait. Despite a non severe drought and some methodological difficulties, lines presenting a good transpiration level under stress were selected to develop new populations with increased resilience to drought.
However, this phenotyping methodology based on IR imaging showed some drawbacks. One is that the method is time consuming. Without possibility to use a wide angle picture, the time required to photograph all the material becomes important for large experiment, thus affecting the comparison between images taken at different moments during the day. Another limit of the methodology is that the micro climatic conditions around the tested material are often not stable. Radiation and wind are fairly variable and have a direct effect on transpiration and canopy temperature. To resolve these limitations it is imperative to correct or standardize the canopy temperature measurements. The better way to do so is to use the Crop Water Stress (CWS) index. Unfortunately in 2009 no adapted equipment was available for us to assess the CWS for standardization of our IR readings.

Future activities

We are already making a new significant improvement in our high throughput phenotyping method for assessing response to water stress conditions. We recently acquired an equipment to assess the CWS that will thus allow standardizing IR readings. The next step in our program is to further combine the use of eco-physiology and molecular markers to increase the efficiency of RS breeding. High quality and high throughput phenotyping, and molecular data will be used for genetic association studies for a better knowledge of the drought adaptation mechanisms.

In addition to our Cirad/CIAT phenotyping project for breeding rice with improved efficiency of water use through RS, we have started a Cirad project entitled “Genetic basis of adaptive diversity in rice; toward tools to pilot selection”. For this project, a set of 181 highly diverse japonica and indica rice will be evaluated for their response to drought and heat stress. We have set-up an experiment in Santa Rosa for high throughput phenotyping of response to a 15-day water stress applied at vegetative stage, using the improved technology we suggested after our previous experiment. Genotyping with SNP markers will be performed on this collection of germplasm and phenotypic and molecular data will then be put together for genome wide association study.

Publications

Audebert, A., Chatel, M., Grenier, C., Ospina, Y., Rodriguez, F. 2009. Breeding for water use efficient rice: toward large scale phenotyping under field conditions for Marker Assisted Recurrent Selection (MARS). Poster presented during the Inter Drought III Meeting: Shanghai-China 11 – 16 October 2009